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Antioxidants in Female Reproductive Biology

Banashree Nath and Hirok Roy

Abstract

Human female reproductive biology is a complex system and its pathologies are varied. However, majority of the pathologic processes involves the role of reactive oxygen species (ROS). Imbalance between the ROS and antioxidants results in oxidative stress (OS). OS is the pathognomonic factor in various female reproductive system ailments. OS contributes to the pathophysiology of infertility, pregnancy related complications, endometriosis, ovarian cancers, etc. Evidence of elevated oxidative stress biomarkers can be found in various inflammatory conditions. Numerous strategies have been postulated for management of OS related pathologic conditions. Antioxidants supplementation may play a crucial in prevention and management of these conditions. However, robust evidence is needed to support the role of antioxidants supplementation in various female reproductive disorders.

Keywords: free radicals, oxidative stress, cellular damage, antioxidants, female reproductive tract diseases

1. Introduction

Oxygen is vital for sustaining life. However, its damaging effect on living cells through production of reactive oxygen species is a paradox of cell metabolism [1]. These free radicals with unpaired electrons are toxins that are produced as the body cells uses oxygen to sustain for processing food or reacting to the environment. They have damaging effect through adverse reactions to various components of the cell [2].

Antioxidants also called “free-radical scavengers”, are biological and chemical substances that fights the damage to cells caused by free radicals produced in our body. These are metabolites and enzymes that either prevent formation of free radicals or clear them from the body before they exert harmful effects on the integral components of cell such as DNA, proteins and lipids [1, 3]. Ineffective elimination of free radicals leads to oxidative stress. Oxidative stress is the pathognomonic factor in various female reproductive system ailments. Diverse effects of free radicals on female reproductive system are subject to location, concentration and the extent of exposure to these molecules [4]. Oxidative stress can affect manifold physiological mechanisms such as oocyte maturation, fertilization, implantation, embryo development and hence contributes to the pathophysiology of pregnancy related complications, endometriosis, polycystic ovarian disease, unexplained infertility and gynecological cancers [5]. The objective of this chapter is to discuss the influence of antioxidants in

different physiological processes to maintain healthy female reproductive function and their role in the prevention of various diseases of the female genital tract.

2. Free radicals

Free radicals are products of normal cellular metabolism with an unpaired electron which makes them highly reactive and unstable [6, 7]. They have high tendency to react with other molecules to initiate a chain of reactions resulting in cellular damage and disease [8]. They are mainly of two types: reactive oxygen species (ROS) and reactive nitrogen species (RNS).

2.1 Reactive oxygen species

Reactive oxygen species (ROS) comprise of mainly three types: (a) superoxide anion (O_2^-), (b) hydrogen peroxide (H_2O_2), and (c) hydroxyl radical ($HO\bullet$) produced due to partial reduction of oxygen [8]. Endogenous sources of free radicals and other ROS in the body originate from the basic reactions of metabolism essential for sustenance of life while exogenous sources are essentially due to hazardous exposure to X-rays, ozone, cigarette smoking, air pollutants, certain drugs and pesticides, and industrial chemicals [9]. Origin of endogenous free radicals may be from: mitochondria, xanthine oxidase, peroxisomes, inflammation, phagocytosis, arachidonate pathways, exercise, ischemia/reperfusion injury [10]. Enzymatic and nonenzymatic reactions both give rise to free radicals in the body. Most of enzymatic reactions occur in respiratory chain, phagocytosis, prostaglandin synthesis and cytochrome P-450 system [11] while nonenzymatic reactions are those of oxygen with organic compounds and ionizing reactions [12].

ROS have been associated with a number of diseases of female reproductive tract as their presence in ovaries, [13–17], fallopian tubes [18] and embryos [19] have been established in various animal and human studies. ROS has been implicated in the regulation of integral functioning of female reproductive organs viz. oocyte maturation, ovarian steroidogenesis, embryo metabolism, corpus luteal function and luteolysis [13, 14, 20].

ROS are formed from normal cellular metabolism and comprises of oxygen ions, free radicals and peroxides. They take part in chemical reactions that remove their unpaired electron. Either an increase in levels of ROS or a decline in the antioxidant defence system of cells causes oxidative stress that induces direct or indirect ROS-mediated macro molecular damage of nucleic acids, proteins, and lipids. This has significant implications in pathogenesis of carcinogenesis [21], neurodegeneration [22, 23], atherosclerosis, diabetes [24], and aging [25]. ROS is assumed to regulate signalling of cellular pathways by which it may contribute to tumour metastasis by gene activation [26]. The knowledge of chemo-interactive processes occurring between the ROS and various molecules involved in the cellular signalling pathways is crucial for understanding the pathogenesis of oxidative stress. Redox reaction between different protein residues and the ROS is the core component of this chemo-interactive process. This redox reaction results in the oxidation of cysteine residues on proteins to form reactive sulfenic acid ($-SOH$). Further oxidation of sulfenic acid ($-SOH$) results in the formation of sulfinic ($-SO_2H$) or sulfonic ($-SO_3H$) acid or sulfenamide (in presence of nitrogen). Oxidation of these protein components results in various ultrastructural changes and/or functional alteration. Some of these alterations are reversible with the aid of antioxidant defence mechanism viz. thioredoxin pathways but rest of the alteration especially involving the generation of sulfonic acid results in permanent damage [2].

2.2 Reactive nitrogen species (RNS)

Nitric oxide-derived compounds, which includes nitroxyl anion, nitrosonium cation, higher oxides of nitrogen, S-nitrosothiols, and dinitrosyl iron complexes are reactive nitrogen species (RNS). RNS are considered to modulate the physiologic processes of many living cells which may include smooth muscle cells, cardiomyocytes, platelets, and nervous and juxtaglomerular cells. Nitric oxide (NO) is formed when L-arginine is converted to L-citrulline by nitric oxide synthase (NOS) [27–29]. NO plays a vital role in modulating diverse physiological mechanisms such as relaxation of arterial and venous smooth muscles and inhibition of platelet aggregation but excess of NO may not have favourable consequences [28, 30]. NO with an unpaired electron is highly reactive and can induce harmful effects to proteins, carbohydrates, nucleotides and lipids. Along with synergistic effects from other mediators of inflammation, this free radical can cause tissue damage, inflammation and adhesions by inducing nitrosative stress [28].

Nitrosative stress has been linked to inflammatory vascular disease in pregnant women such as preeclampsia where the pathognomonic features include hypertension along with generalized endothelial dysfunction, proteinuria, and foetal growth restriction [31]. There is surge in production of nitric oxide due to vascular inflammation induced by shed membrane particles of leukocytes and platelets along with elevated levels of microparticles from plasma membrane of apoptotic or activated circulating cells or cells of the vascular wall [32, 33] resulting in generation of pro-inflammatory proteins. Platelet microparticles more precisely are pro-inflammatory and hence contribute markedly for nitrosative stress in the vascular wall [34].

3. Antioxidants

To combat the adverse effect of oxidative stress, human body has evolved various defense mechanisms viz. preventive and repair mechanisms, physical defenses, and antioxidant system. Antioxidants can be chemically described as any molecule that can donate electron/s or act as a reducing agent in a redox reaction resulting in conversion of a reactive molecule in to a relatively stable and inert substances. Antioxidants by its virtue as an electron donor can react with ROS, resulting in suppression of oxidative stress [35]. However, this simple way to describe “antioxidants” cannot be justified and it has much wider connotations and complexity. The ability of a molecule to participate in a redox reaction with a ROS is not only the distinguishing feature for an antioxidant. There are numerous molecules which can interact with a ROS resulting from the modulation of inflammatory cascades. Inhibitor molecules for NADPH oxidase and lipoxygenases as well as cofactors for antioxidant enzymes, and metal chelators which can negate the ROS levels, can also be termed as antioxidants. However, it should be remembered that in an appropriate environment, many of these antioxidants may function as a prooxidant due the inherited redox potential of these substances [36, 37]. Hence, proper understanding of these diverse molecules is critically needed prior to formulating rational antioxidant therapies. Antioxidants can broadly be classified as enzymatic and non-enzymatic as depicted in **Table 1**.

Antioxidants, both endogenous and exogenous in origin, counteracts free radicals mediated injury in an interactive and synergistical ways (**Table 2**) [38].

3.1 Enzymatic antioxidants

Enzymatic antioxidants are endogenously synthesized molecules and are more efficacious as free radical scavenging role. These antioxidants have transition

Enzymatic antioxidants	Non-enzymatic antioxidants
Superoxide dismutases (SOD)	a. Endogenous Antioxidants
Peroxidases	• Bilirubin
Catalases	• Uric acid
Thioredoxin (Trx) system	• NADPH and NADH
	• Thiols, e.g., glutathione, N-acetyl cysteine
	• Ubiquinone
	b. Dietary Antioxidants & micronutrients
	• Vitamin A, C, E and the B complex, Zinc, Selenium
	• Beta carotene and other carotenoids
	• Polyphenols, e.g., flavonoids, flavones
	c. Metal Binding Proteins
	• Albumin (copper)
	• Ferritin (iron)
	• Myoglobin (iron)
	• Transferrin (iron)
	• Metallothionein (copper)
	• Ceruloplasmin (copper)

Table 1.
Classification of antioxidants.

ROS	Neutralizing antioxidants
Hydroxyl radical	Vitamin C, Glutathione, Flavonoids, Lipoic acid
Superoxide radical	Vitamin C, Glutathione, Flavonoids, Superoxide dismutase
Hydrogen peroxide	Vitamin C, Glutathione, beta carotene, Vitamin-E, flavonoids, lipoic acid
Lipid peroxides	Beta-carotene, Vitamin-E, Ubiquinone, flavonoids, Glutathione peroxidase

Table 2.
ROS and Neutralizing Antioxidants.

metal core which are capable of transfer of electrons essential for redox reaction with ROS and thereby, effectively neutralizing the adverse effects of ROS. These endogenously produced enzymes include superoxide dismutase enzymes (SODs), glutathione peroxidase, catalases, and glutathione oxidase.

Superoxide dismutase: SOD is an enzyme system that catalyzes the of the superoxide (O_2^-) radical into ordinary molecular oxygen (O_2) and hydrogen peroxide (H_2O_2). SODs are group of metalloproteins and its enzymatic functions were first discovered by Irwin Fridovich and Joe McCord at Duke University in 1968 [39]. There are various isoforms of this enzyme but in humans, it exists mainly in three isoforms viz. SOD1, SOD2 and SOD3. SOD1 is located in the cytoplasm, SOD2 is predominantly found in the mitochondria, and SOD3 is extracellular. The genes are located on chromosomes 21, 6, and 4, respectively (21q22.1, 6q25.3 and 4p15.3-p15.1). SOD1 is a dimer, whereas SOD2 and SOD3 are tetramers. SOD1 contains a core of two metal cofactors comprising of copper (Cu) and zinc (Zn). Mitochondrial SOD2 contains manganese and is encoded by nuclear DNA. SOD3, the extracellular enzyme, Cu-Zn has in its reactive center.

Glutathione peroxidase: It belongs to a large class of enzyme system called peroxidases. The primary biochemical function of glutathione peroxidase (GPx) is the conversion of lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water, which is the crucial function as an antioxidant. GPx exists in eight different isoforms in humans and contains selenium as its reactive core. It is a tetrameric enzyme with the exception of GPx4 which is a monomeric enzyme.

Catalases: It is an iron containing enzyme which catalyzes the biotransformation of hydrogen peroxide to non-reactive water and oxygen.

Apart from these endogenous enzymatic antioxidants, thioredoxin plays a central role in humans as a defensive response to ROS. They act as antioxidants by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange. They also play an important role in cell survival [40].

3.2 Nonenzymatic antioxidants

Nonenzymatic antioxidants comprises of various exogenous dietary nutrients as well as endogenous compounds. They may act directly as an antioxidant or as an adjuvant to others molecules which participates in redox reaction.

Vitamin C and vitamin E plays a central role as defense mechanism against ROS. They act synergistically as a redox buffer to reduce the impact of oxidative stress imposed by various ROS. Vitamin C neutralizes superoxide radicals as well as other singlet oxygen species. Vitamin E is a potent scavenger of peroxy radicals. α -tocopherol has the most potent antioxidant capabilities compared to other tocopherols due to the fact that H⁺ donating ability of different tocols increases in efficiency with greater ring methyl substitution. Thiols like glutathione is another endogenous antioxidant found in humans and is also can be found in oocytes and embryos in abundant amount. They exert their effect by virtue of its thiol component of cysteine residue which take part in the redox reaction. Moreover, glutathione can act as a cofactor for several antioxidant enzymes viz. glutathione peroxidase (GPx) and glutathione transferase [41]. Various micronutrients like Zn, Se, Cu aids the enzymatic antioxidants to counter the ill-effects of OS. Metal binding proteins like transferrin and ceruloplasmin combines with free iron ions which is a vital component of redox reaction (Fenton). Bilirubin is a tetrapyrrole pigment which possesses potent antioxidant and anti-inflammatory properties. This remarkable antioxidant activity appears likely to stem largely from the inhibitory action of unconjugated bilirubin over common isoforms of NADPH oxidase, which is an active participant of superoxide-generating reactions [42–44]. Another endogenous molecule bearing potent antioxidant property is uric acid. Uric acid is an efficient oxygen radical scavenger. At physiological concentrations, urate reduces the oxo-heme oxidant formed by peroxide reaction with hemoglobin, thereby, protecting the erythrocytes from lysis due to lipid peroxidation.

4. Female reproductive diseases

The prooxidants and antioxidants work in synchrony to maintain a balance in the milieu of cellular metabolism. When this mechanism fails, oxidative stress is the sequelae. Prooxidant generation is influenced by cytochrome P450, and the corpus luteum is presumed to be one of its key sources [45]. The crucial role of free radicals in regulating the various physiological reproductive processes renders its influence on the oocytes, sperm, and embryos and their microenvironments. These changes in microenvironment has a direct bearing on follicular fluid, hydro salpingeal fluid,

and peritoneal fluid which substantially determine the oocytes quality, interaction between sperm and oocyte, implantation of embryo and its early development [46–48]. Hence oxidative stress can affect the prospects of a favourable pregnancy and is also attributed to pathophysiology of endometriosis, hydrosalpinges, polycystic ovary syndrome (PCOS), and unexplained subfertility [49].

4.1 Female subfertility

World Health Organization (WHO) defines Infertility or subfertility as the “failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse” [50]. With 48.5 million couples infertile globally [51], it is a health concern spread worldwide in which female factors are assumed to contribute to 40–50% percent of cases [52, 53]. Ovulatory dysfunction, tubal and pelvic pathology, endometriosis, and poor egg quality are among the most common causes of female subfertility while reproductive age and body mass index (BMI) are vital demographic determinants [54]. Notwithstanding the evidence of obvious pathologies, role of toxic products produced during oxidative stress in the occurrence of female subfertility is well established [55, 56].

ROS however has dual role. It may serve as key modulating agent in numerous physiological processes while in excess may cause cellular damage. The equivalence in the body is maintained by appropriate levels of antioxidants and hence determining the levels of the antioxidants as total antioxidant capacity (TAC) has been investigated in many studies [57–59]. Ovulation is one of key physiologic process in female that is evidenced by high numbers of macrophages and neutrophilic granulocytes in the follicle wall along with fast metabolism of granulosa cells. This gives high probability of ROS generation which may be essential for oocyte development and consequent progression to embryo. ROS are assumed to influence oocyte maturation [60] luteolysis [61, 62] progesterone secretion by the corpus luteum [63, 64] ovulation and follicular atresia [65]. ROS are linked to single follicle generation by stimulating atretic regression of the cohort of newly grown follicles through its action on granulosa cells to exhaust its reactivity to gonadotrophic hormones and steroidogenic function [65]. Pasqualotto et al. [66] found positive correlation of lipid peroxidation (LPO) and total antioxidant capacity (TAC) in pooled FF to pregnancy rate hence concluding high levels of LPO do promote oocyte and embryo development and enhance their quality.

Several studies have documented benefits of supplementary antioxidants in such cases with diverse methods of action. Vitamin E is assumed to improve epithelial growth in blood vessels and in the endometrium [67]. *N*-acetyl-cysteine improves cervical mucus for sperm penetration and also helps in ovulation [68]; L-arginine increases endometrial blood flow for successful implantation [69]; Myo-inositol improves ovarian function by decreasing levels of androgen and increasing sensitivity to insulin [70] while PUFAs promotes prostaglandin and steroid hormone synthesis apart from contributing to formation of cell membranes of the sperm and oocyte essential for fertilisation [71]. Even in Assisted reproductive technology, ROS may arise from cumulus cells, leucocytes, and culture media. Hence IVF cycles will yield better results if patients are screened for oxidative stress levels [72]. Culture medium are supplemented with antioxidants like β -mercaptoethanol, protein, vitamin E, vitamin C, cysteamine, cysteine, taurine and hypotaurine, and thiols to enhance the growth and maturation of embryos by downplaying the effects of ROS. ROS are scavenged by antioxidants and subsequently exert favourable effects by reducing blastocyst degeneration, embryo apoptosis and increasing hatching of blastocysts. Improvement of sperm morphology and preimplantation embryo development as well as reduction of developmental defects are reported with inclusion of antioxidants in the treatment protocol of infertile patients [73].

4.2 Endometriosis

Endometriosis is defined as the presence of endometrial tissue outside of uterine cavity which lead to chronic inflammatory reaction [74]. It affects 6–10% of women in the general female population and is prevalent in 30–45% of patients with infertility or chronic pelvic pain [75, 76]. It is one of the most widespread gynaecological diseases and women present mostly with significant cyclic pelvic pain that escalate just prior to starting of menses and subside with onset of blood flow. In a few atypical cases, patients may not have any complaint and hence poses a dilemma for diagnosis [77]. This oestrogen dependent female pelvic pathology is mainly diagnosed by laparoscopy or laparotomy and confirmed by histopathological examination of retrieved pelvic tissues [78].

There are innumerable theories of the pathophysiology of endometriosis. The origin of OS in endometriosis is assumed to be induced by apoptotic endometrial tissue which get implanted in peritoneal cavity through retrograde menstruation. This tissue along with menstrual effluent are assumed to be antigenic and activate macrophages. Consequently, the number and activity of macrophages in the peritoneal fluid (PF) increase that phagocytose antigens. This results in release of inflammatory cytokines such as interleukin (IL) 2, 4, 10, TNF- α and IFN- γ [79, 80] and serum and peritoneal IL-33 in cases of deeply infiltrating endometriosis [81]. Increased inflammatory activity is known to cause OS [82]. Apart from macrophages, there is upregulation of transcription factor NF- κ B in endometriosis [83–85] which can activate many genes to further increase inflammation and promote progression of the disease [86]. ROS damage the fragile mesothelium thus facilitating adhesion and implantation of the endometrial cells to further promote progression of the disease [87]. Hence elevated Oxidative stress could be cause or a consequence of one of this debilitating diseases of females of reproductive age group [8].

Despite endometriosis being a disease with benign pathology, there are features in endometriosis identical to cancer such as the invasion of tissues, tendency to evade programmed cell death, distal spread and high propensity of angiogenesis. [88] which renders its close association to ROS reported in numerous studies [89, 90]. The essential feature of angiogenesis and immunity to apoptosis common to both endometriosis and tumorigenesis results in accelerated proliferation rate subsequently producing increased ROS. The pathogenesis of cellular damage by ROS in both endometriosis and cancer cells is same. ROS acts as a second messenger of cell proliferation [91] by activating MAPK signalling pathways to accelerate cell proliferation. Hence this linkage between ROS and cell proliferation in cancer illustrates the definite role of elevated ROS in modulating cell proliferation in endometriosis. To elucidate the fact, Ngô et al. [91] used purified stromal and epithelial cells from ovarian endometrioma and ectopic endometrium of endometriosis to create cell lines. The experiment showed endometriotic cells had high OS levels with increased ROS production and decreased catalase levels. There was increased cellular proliferation and activation of ERK1/2. A group of enzymes called MMPs also contribute to increase ROS in the pathogenesis of endometrial disease. MMPs facilitate implantation of ectopic endometrial cells by degrading the extracellular matrix of the peritoneal mesothelium [92]. Hence the recent strategies to reduce oxidative stress for effective treatment of endometriosis in preference to creating non-oestrogen environment by pharmacological intervention has been gaining evidence. Studies showed there is decrease in symptoms with antioxidant (vitamin E, vitamin C, zinc and selenium) intake [93] as well as improvement in antioxidant markers with antioxidant rich diet [94]. Aminoguanidine through OS reduction prevent adhesion to peritoneal surface.

There are a number of compounds that can act as antioxidants and can manifest its favourable effects in improving the symptoms of endometriosis.

Vitamin C is required for carrying out diverse physiological processes of the body. Humans and other primates acquire it from exogenous sources that acts as co-factor for a number of enzymes, most notably hydroxylases involved in collagen synthesis apart from being an effective antioxidant. Vitamin E is a lipid-soluble antioxidant that scavenges peroxy radical to eliminate the effect of free radicals and get transformed to tocopheryl radical that is subsequently reduced by a hydrogen donor (as Vitamin C) to return to its reduced state [95].

Resveratrol is a natural polyphenolic flavonoid having antineoplastic, anti-inflammatory and antioxidant properties [96]. It is synthesized by plants following exposure to ultraviolet radiation. Its anti-inflammatory effect is mediated through inhibition of cytokines (tumor necrosis factor- α , IL-6, IL-8), VEGF and monocyte chemotactic protein 1. It inhibits production of ROS by monocytes, macrophages and lymphocytes and regulate cell proliferation and apoptosis by inhibiting NF- κ B [97]. It reduces VEGF levels to prevent angiogenesis [98]. Resveratrol is already used in the treatment of several clinical conditions such as cardiovascular diseases, cancer, type-2 diabetes mellitus and neurodegenerative diseases and is being explored for its impact in the treatment of endometriosis.

Melatonin is secreted by pineal gland predominantly during the night and has potent antioxidant effects. It is also shown to stimulate antioxidant enzymes [99]. It can induce down-regulation of MMPs. Hence its mode of action as antioxidant in endometriosis can range from scavenging toxic free radicals [100] to regulating levels of MMPs in ectopic and eutopic endometrial tissue [101, 102]. It can also act as antioxidant enzymes stimulator [99].

N-acetyl-L-cysteine (NAC) is another antioxidant which is precursor of glutathione [103] and is found to inhibit proliferation of endometrial cells [104, 105]. It acts through its ability to limit tissue invasion [106] and suppression of NF- κ B activation which acts as a transcriptional factor in the pathophysiology of endometriosis [107]. Its other mode of action is linked to activation of the immune system by increasing IL-2 levels and the expression of CD25 on T cells [107] apart from expression of membrane TNF- α expression [103].

Xanthohumol is extracted from *Humulus lupulus* (species of flowering plant of Cannabaceae) and has antioxidant and anti-inflammatory properties. Rudzitis-Auth et al. suggested Xanthohumol if taken through dietary supplements is effective for selective treatment of endometriotic lesions [108]. It acts by inhibition of NF- κ B signaling pathway [109] as well as decreasing production of NO by through suppressing of NO synthase [110].

Epigallocatechin-3-gallate (EGCG) is another antioxidant found in green tea which has also antimitotic and antiangiogenic properties [111]. The effect of EGCG on endometriosis is studied both in vitro and in vivo and is assumed to reduce OS by VEGF reduction and inhibition of angiogenesis. It also inhibits cell proliferation, migration and invasion of endometrial cells [112].

4.3 Pre-eclampsia

Pre-eclampsia involves gradual development of hypertension (systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg), or deterioration of pre-existing hypertension, proteinuria (300 mg/L or more in 24 h), generalized oedema, and sometimes blood clotting disorders occurring after 20 weeks of gestation [113]. Severe preeclampsia may progress to eclampsia and the HELLP syndrome, which involves haemolysis, elevated liver enzymes and low platelets. The spectrum of these hypertensive disorders can impose serious foetal morbidity like

intrauterine growth restriction and preterm births which has further implications of early neonatal death and infant mortality. The pathophysiology of pre-eclampsia still remains a subject of research. The existing theories suggest incomplete cytotrophoblast invasion into the spiral arteries in the uterus that causes malformed placenta leading to deficient perfusion of placenta. Role of antiplatelet agents and calcium supplements to delay this process are being studied [114, 115]. This inadequate placental perfusion may induce OS with decline in concentrations of antioxidants in plasma and placenta [116, 117]. This complemented with inflammatory response of body cause damage to endothelial cells resulting in signs and symptoms of pre-eclampsia. Antioxidants prevent oxidation of proteins and enzymes, destroy free radicals, and preserve the integrity of cellular membrane [118]. Hence dietary or therapeutic antioxidant supplements in women can possibly eliminate the deleterious effects of OS and uteroplacental endothelial damage evident in preeclampsia. A few recent meta-analysis however failed to show any beneficial role of antioxidant therapy on prevention of PE [119, 120]. More trials are needed to establish the effect of antioxidants on pre-eclampsia and to provide evidence-based information on its adverse effects and long-term consequences on the growth and development of the children.

4.4 Peripheral neuropathy in obstetrics

Pregnant women are prone to a number of neuropathic syndromes due to physical changes resulting from enlargement of the uterus and the gradual growth of the foetus. The subsequential postural changes and nutation of the pelvic girdle and vertebrae due to high concentrations of relaxin released from the tenth week of gestation gives rise to low back pain and entrapment neuropathies. Analgesic drugs and surgery being contraindicated in pregnancy, the best approach to treat such morbidity in pregnancy remains a dilemma. Rehabilitation therapies and neuro-protectors in such cases have proved effective in relieving pain and paraesthesia of peripheral neuropathies [121]. Role of antioxidants as neuroprotective agents are investigated lately. One of such agent is alpha-lipoic acid (ALA) which acts as a powerful antioxidant in the body. It exerts its functions in every cell and tissue in the body due to its solubility in both water and fat. This potent antioxidant can modulate many inflammatory pathways through its inhibitory effect on production of vascular and intracellular adhesion molecules (VCAM-1 and ICAM-1), reducing the expression of CD4 on blood mononuclear cells, secretion of tumour necrosis factor (TNF)- α and inhibition of natural killer (NK) cells [122]. It also has direct inhibitory action on the transcription factor NF- κ B which regulates the expression of various genes associated with inflammatory response and cell apoptosis [123]. Hence supplementation with ALA by virtue of its antioxidant and anti-inflammatory action has been shown to relieve pain and paraesthesia in patients with sciatica, carpal tunnel syndrome and diabetic neuropathy [124–127].

ALA supplementation at doses of up to 2400 mg/day and intravenous administration of 600 mg/day seemed to be safe in humans [123]. The safety of this potent antioxidant in pregnant women are yet to be established, however several studies have credited its role in the prevention of premature rupture of membranes, threatened miscarriage and gestational diabetes [128].

4.5 Gynecological (ovarian, endometrial and cervical) cancers

Gynaecological cancers present a diagnostic challenge in early stage since the symptoms appear mostly when the disease has metastasized. Two third cases are diagnosed in advanced stage accounting for high fatality-to-case ratio among all

malignancies in women [129]. The process of carcinogenesis occurs in multiple steps starting from normal cell to pre-cancerous stage and finally to an early stage of cancer [130]. A single cell undergoes sequential events in the form of initiation, promotion and progression to finally turn cancerous. ROS can act in all the three stages to stimulate carcinogenesis [131].

Initiation is the first stage of carcinogenesis and is executed as a consequence of an irreversible genetic alteration, that may be either simple mutations, transversions, transitions, and/or small deletions in DNA. DNA damage by oxidation can also occur through hydroxyl radical produced from H_2O_2 . Increased oxidative stress may deplete the endogenous antioxidant reserves and activate endonucleases resulting in DNA fragmentation [130]. The second stage of promotion is reversible and it mediates promoter-receptor interactions to influence expression of the genome. Tumour promoters induce production of oxygen radical by modulating metabolic processes of cells. These oxygen radicals through modulation of gene associated with proliferation or cell death can induce the expression of mutated cell clones. However low levels of oxidative stress can promote cell division and tumour growth whereas high levels have cytotoxic effects and can hinder cell proliferation [131, 132]. In the ultimate stage of progression there is karyotypic instability and malignant growth. Progression is characterized by expeditious proliferation of cells, elusion from immune suppression, tissue invasion and metastasis [133]. Genesis of abundant free radicals along with increase in the level of oxidized DNA bases during oxidative stress may incite some tumours to mutate, inhibit anti-proteases and cause damage to local tissues [134]. In fully developed cancer cells, these modified DNA bases may induce genetic instability and metastatic propensity [135].

Diverse natural substances have been recognised to have role in cancer prevention [136] and treatment [137] when supplemented with chemotherapy, radiotherapy, and surgery. The drugs used in radiotherapy and chemotherapy can cause diverse tissue and organ damage through generation of free radicals which can also induce carcinogenesis [138]. Several studies reported that using antioxidants with anticancer drug therapy can significantly decrease these cellular damages and is associated with reduced risk of all-cause mortality and recurrence risk [138, 139].

However, many natural antioxidants may depict conflicting properties in cancer cells. The actions vary according to their concentration. With the possibility of antioxidants causing direct damage to DNA and the cell, speculations are arising on the role of antioxidant in the causation of cancer [140]. Recent studies suggest supplementation of antioxidants in high doses may have ill effects on health. The association of high doses of beta-carotene with lung cancer in smokers and high doses of vitamin E with that of prostate cancer are some examples of health hazards of antioxidants [141].

Hence the biological characteristics of natural antioxidants are assumed being beneficial or harmful depending on their concentration. The natural substances exhibit their antioxidant potential in lower concentration by increasing the expression of ROS scavengers while higher concentration can generate oxidative stress.

4.6 Polycystic ovarian disease

Polycystic ovary syndrome (PCOS) is a major public health disorder affecting 5–21% of women in reproductive age group. This most common endocrine disorder among reproductive-aged women has multitude of manifestations that includes reproductive (hyperandrogenism, hirsutism, anovulation, infertility, and menstrual disturbance), metabolic (obesity, diabetes mellitus and cardiovascular risk), and psychological (mood disorders and decreased quality of life) components [142]. Several studies undertaken still fail to unravel the pathophysiology of PCOS

with theories attributing the clinical phenomenon to oxidative stress, inflammation, endothelial injury and genetic mechanisms. Markers of OS are considerably elevated in patients with PCOS and are assumed to play significant role in its pathogenesis [143]. NAC (N-acetyl-cysteine) is an antioxidant which can influence insulin receptor activity and its secretion [144] and also has negative effects on apoptotic activity and can lower homocysteine levels [145]. NAC has shown favourable effects on ovulation induction in PCOS women and can be used as an adjuvant to drugs for ovulation induction [146].

4.7 Diabetes in pregnancy

Gestational diabetes mellitus can pose risk for subsequent development of later-onset DM, metabolic syndrome, and cardiovascular disease [147]. Prevalence of diabetes is nearly 1–14% of pregnancies [148, 149]. Diabetes is a disease condition that could result in production of free radicals through glucose oxidation, alterations in antioxidant defence system, lipid peroxidation, non-enzymatic glycation of proteins and oxidative destruction of glycated proteins [150–151]. Marked deficiency in antioxidant activity have also been reported during the development of gestational diabetes [152]. Levels of antioxidants such as selenium, zinc, and vitamin E are reported to be reduced in GDM [152, 153]. Even adequate intake of antioxidants through diet improves total antioxidant status (TAS) and overall health condition [154].

There are several means by which elevated blood glucose levels induce excess production of ROS [154–156]. High glucose levels during pregnancy can also bring teratogenic changes in foetus through chemical modification and complex rearrangements of DNA. These are brought about by glycation products from elevated glucose levels hence reflecting the significance of genomic injury that can disturb embryonic development. The deficiency states of membrane lipids, changes in biological prostaglandin cascade and the formation of excess free radicals bring about dysmorphogenesis in fetus in diabetic pregnancy [157]. There is decrease in antioxidant defence in women with GDM due to accelerated lipid peroxidation causing membrane damage. Lipid peroxidation generate hydroperoxides that alters prostaglandin cascade of biosynthesis and may contribute to the morbidity by impairing the antioxidant defence system [158, 159]. GDM also induces oxidative stress in the foetus. Hence Increasing antioxidant intake during pregnancy remains an important part of promoting health status during pregnancy. Ahmed M. Maged *et al* reported lowered use of insulin dose for control of blood sugar with antioxidants supplementation in women with GDM. There was markedly improved neonatal outcome with decrease in NICU admission and less cases with RDS [160].

4.8 Abortion

Normal early pregnancy is subject to increase in oxygen concentration with more chances of ROS formation. This occurs from enzymes of respiratory reactions where electrons leak within the mitochondria. The syncytiotrophoblast by virtue of its position on the villous surface is highly vulnerable to OS due to direct exposure to high intervillous PO₂. The syncytiotrophoblast consequently suffer loss of function and degeneration. The vulnerability is also high due to lower concentration of antioxidant enzymes in the syncytiotrophoblast when compared to other villous tissues during early pregnancy. Even high levels of antioxidants needed to neutralize and scavenge excessive ROS in women with recurrent abortion contributes to its pathogenesis apart from free radicals formed during the process. Studies reported there is elevated levels of lipoperoxides and significantly decreased vitamin A, E,

and beta carotene in women with recurrent pregnancy loss thus indicating that OS may be implicated in the occurrence of recurrent abortion. Studies further reported deficient glutathione peroxidase activity in women with recurrent abortion compared to non-pregnant woman or healthy pregnancies [161–163]. Even concentration of selenium in the hair samples of women with recurrent abortion were found to be significantly lower when compared to healthy viable pregnancies [164].

With many studies affirming OS in the pathogenesis of recurrent abortion, supplementation of antioxidants during pregnancy have gained importance. Optimum dietary intake of antioxidants like vitamins C, E, and A, lycopenes, selenium compounds, lipoic acid, and ubiquinones have a role in the prevention of cellular damage and hence in the occurrence of recurrent pregnancy loss by scavenging ROS. Vitamin C and E are two antioxidants most explored and suggested to have beneficial role in scavenging free radicals and hence reduce the effects of OS in women with pregnancy loss. Both vitamins have been found to increase in normal pregnancy and decrease in conceptions with complications implying their use and consumption while balancing the OS found in such circumstances. However, the determination of appropriate dose and type of antioxidant is essential to prove its favourable effects without causing any harmful effects on the mother or foetus [165, 166].

5. Conclusion

Free radicals and oxidative stress are assumed to influence diverse physiological functions in reproduction, as well as in conditions such as infertility, endometriosis, PCOS, abortion, hydatidiform mole, foetal embryopathies, peripheral neuropathy of pregnancy and other pregnancy complications such as GDM, IUGR and pre-eclampsia. In-vivo evaluation of oxidative stress is still not accurately possible to assess. With many studies reporting role of OS in the physiological processes of reproductive tract, the lowest essential measure of ROS for sustenance of life which is well tolerated need to be specified. Extensive research on the role of different biomarkers in predicting the effects of ROS in averting the complications discussed above shall prove valuable. The effects of antioxidant therapy in preventing complications in deficient and sufficient individuals is indeed subject of research.

Conflict of interest

The authors declare no conflict of interest.

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Author details

Banashree Nath^{1*} and Hirok Roy²

1 Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Raebareli, Uttar Pradesh, India

2 Department of Anaesthesiology, All India Institute of Medical Sciences, Raebareli, Uttar Pradesh, India

*Address all correspondence to: nathbanashree@gmail.com

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